Factors Affecting Drip Loss from Thawing Thornless Blackberries

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Abstract. **Fruits from thornless blackberry (*Rubus* sp.) cultivars were compared to determine causes of variation in drip losses during thawing after frozen storage. Drip was similar in composition to juice obtained by pressing. Drip losses for different cultivars ranged between 1% and 30% in 1984; increased losses in 1983 were attributed to poor fruit condition (e.g., deterioration during postharvest holding). Drip losses were greater in riper samples but did not depend on fruit size. Drip losses were correlated with low insoluble pectin. Microscopic examination revealed an inverse relationship between the tendency to drip and the epidermal cell layer thickness.**

In a previous study of the composition and processability of thornless blackberries, we observed that the frozen fruit released large and variable quantities of pigmented exudate during thawing (18). The tendency of some cultivars to incur excessive drip losses may limit their suitability for commercial or home freezing. However, information on the extent and cause of cultivar variation in drip losses for blackberries is very limited. Crivelli and Rosati (3) reported consistent cultivar differences in drip losses over two seasons, the losses being between 4% and 7% for two thornless blackberry cultivars and between 4% and 32% for 10 raspberry cultivars. The extent of drip with blackberries and raspberries appears to depend more on raw material condition than on freezing or thawing methods (12, 19). Studies with strawberries suggest that cultivar differences in suitability for freezing may be related to the size of parenchyma cells—small cells being less subject to rupturing than large (1). Calcium–pectin interactions also may play a role in the capacity of fruits to withstand freezing and thawing. It is well-known that Ca binding to pectic substances increases the cohesion of plant cell walls (5). The contributions of pectins and Ca to the texture of fresh and frozen fruit are well-established (2, 17). Calcium addition to strawberries prior to freezing has been shown to firm the thawed product (15) and reduce drip losses (4).

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Our objectives in this study were to determine the extent of drip loss with the principal named cultivars and some promising selections of thornless blackberries and to identify the structural and compositional factors associated with minimal drip loss.

Materials and Methods

Source and preparation of blackberry samples. Thornless blackberry samples weighing 1-2 kg were obtained from the USDA Beltsville Agricultural Research Center in 1983 (six cultivars and six selections) and 1984 (five cultivars and four selections). The selections compared were C-33, C-57, C-58, C-61, and C-62 (all from SU-IS 50 x 'Thornfree'); C-67 (62-76-7 x 64-21-11); 64-9-3 (SU-IS 3 x 'Thornfree'); and 64-21-3 (SU-IS 47 x 'Thornfree'). The samples, which consisted of both shiny black and dull black fruit, the latter being fully ripe (22), were transported within 4–5 hr of harvest to our laboratory in Philadelphia in an insulated container cooled with ice water. After overnight storage at 1°C, the samples were sorted, washed, drained, and packaged in polyethylene freezer containers (25 × 29 × 7 cm). Samples were frozen and stored at −13° for 7 to 9 months. During frozen storage, some of the blackberries turned red, a phenomenon previously reported by Jennings and Carmichael (9) and attributed by them to an intracellular pH change in fruit that were not fully ripe. Before samples were taken for evaluation, the individual frozen blackberries were sorted into two subsamples: those fruit that had turned red, and the remaining fruit that had retained their black color. In several cases, clones were represented by only a single subsample since insufficient numbers of fruits of the other subsample were available for evaluation.

Determination of drip in thawing blackberries. About 45 g of frozen blackberries, showing no visual evidence of adhering frozen exudate or physical damage from compression, were weighed into tared 7.62-cm (3-inch) diameter No. 8 stainless steel sieves (W.S. Tyler, Inc. Mentor, Ohio). The number of fruit in each sieve was recorded so that the mean fruit weight could be calculated. The sieves were placed in 100-mm-diameter glass funnels draining into 25-ml graduated cylinders. Drip was collected over a 3-hr period, at the end of which the sieves were tilted and gently tapped to permit droplets adhering to the fruits and/or screen to coalesce and drain. The drip volume and drained weight of the fruit were each measured, and the drip loss was calculated as the drip volume or loss in weight during thawing divided by the weight of the frozen sample. Drip loss determinations were made in quadruplicate for each blackberry sample. The extent of drupelet collapse in the drained fruit was observed by the senior author.

Drip and fruit composition. The soluble solids and total anthocyanin contents of drip samples and of blackberry juice, prepared by pressing thawed fruit in a Carver press (18), were determined for blackberries obtained in 1983. The soluble solids content was determined with a Bausch & Lomb Abbe–3L Refractometer and corrected to 20°C. The total anthocyanin content was determined by the pH differential method of Fuleki and Francis (8) and calculated as absorbance units (A) per milliliter. Blackberry samples were characterized by their soluble solids : titratable acidity ratios (SS:A), as described previously (18).

Soluble and insoluble Ca in blackberry samples were determined by atomic absorption spectrophotometry. Duplicate 50-g portions of fruit were pressed with a Carver press (18) to obtain juice, which was clarified by adding 1.8 g Celite Analytical Filter Aid (Fisher) and filtering through Whatman no. 2 paper under suction. Filtrates were diluted with double-deionized water (DDW) and analyzed for soluble Ca with a Perkin–Elmer Model 306 atomic absorption spectrophotometer at 211 nm (Ca–Mg hollow cathode tube operated at 15 mA). Additional soluble Ca in residual juice adhering to the presscakes after pressing was determined by homogenizing the weighed presscakes with 3 parts DDW in a stainless steel semi-micro blending container on a Waring base for 1 min at high speed, diluting duplicate 5-g portions of homogenate 1:20 with DDW, adding 1.8 g of filter aid, filtering through Whatman no. 5 paper under suction, and analyzing the filtrates for Ca. The soluble Ca content of blackberry samples was calculated as the sum of the juice and water extractable presscake Ca, both expressed on a fruit-weight basis. To determine insoluble Ca, duplicate 5-g portions of homogenate were digested by adding 10 ml HNO_3 and 100 ml DDW and heating on a steam bath for 6–8 hr. After digestion, the volumes of the cooled digests were measured (to compensate for evaporation), the digests were filtered through Whatman no. 42 paper under suction, and the filtrates were analyzed for Ca (soluble + insoluble). The soluble Ca content of each presscake was subtracted from these values to obtain the insoluble Ca content, which was then expressed on a fruit-weight basis. Total Ca in the blackberry samples was calculated as the sum of the soluble and insoluble Ca contents.

Soluble and insoluble pectic polysaccharides were extracted from the acetone-insoluble residues of duplicate 50-g fruit samples with 0.1 M EDTA in 0.2 M Tris·HCl (pH 7.2) at 20°C followed by 0.1 M EDTA in 0.2 M Na_2HPO_4 (pH 6.9) at 95°, according to the procedure of Knee (11). Pectins in the extracts were determined by the *m*-hydroxydiphenyl colorimetric method of Kintner and Van Buren (10) and expressed as percentage of galacturonic acid (fresh weight). Acetone-insoluble residues were prepared without the addition of Teepol and octanol, since these substances interfered with the colorimetric analysis.

Microscopy. Segments of drupelets were obtained with a sharp razor blade by making a shallow cut along the surface of the intact fresh or frozen blackberry fruit. Tissue was fixed in 3% glutaraldehyde in 0.08 M sodium cacodylate buffer, pH 7.0, for 6 hr and then cut into 2-mm pieces and rinsed with the same buffer for at least 30 min. Fixation was continued with 1% osmium tetroxide in the same buffer for 2 hr. Tissue then was dehydrated in a graded acetone series and embedded in Spurr's low-viscosity resin. Sections for light microscopy were prepared at a thickness of 1 mm and stained with methylene blue for contrast enhancement. Dimensions of structural features were measured with an eyepiece micrometer.

Statistical analyses. The combined effects of cultivar and fruit color and their interaction (26 treatments in 1983 and 15 in 1984) on drip loss and compositional variables were investigated each year by analysis of variance. The Bonferroni LSD (13) was used to separate means. Correlations between the drip loss and compositional variables were computed separately for each year and for the red and black subsamples as well as the combined subsamples. All statistical computations were performed with the Statistical Analysis System General Linear Model Procedure (SAS Institute, Cary, N.C.).

Results and Discussion

Characteristics of thawing blackberry fruits. Thornless blackberry samples from the 1983 season lost up to one-third of their weight as drip during thawing under standardized conditions (Table 1). Drip losses could be estimated more accurately by measuring the drained weight rather than the drip volume, especially with samples having losses <10%, because of difficulties in recovering and measuring small drip volumes. Consequently, only gravimetric estimates of drip loss (weight loss × 100 ÷ weight of frozen sample) were compared in our study of cultivar, ripeness, and composition effects on thawing behavior.

In samples with large drip losses (i.e., >20–25%), many of the individual drupelets of thawing blackberry fruits were collapsed, giving these samples an unattractive appearance. Although drupelet collapse generally was increased in samples showing greater drip loss, one clone, 'Chester', which was subject to a very large drip loss, retained its fresh appearance with little drupelet collapse during thawing. In situations where the presence of drip is not objectionable, i.e., toppings for frozen desserts, this clone might be acceptable for freezing.

The drip from all blackberry samples was highly colored and had the same total anthocyanin and soluble solids contents as juice obtained by pressing (Table 2). The large drip volumes observed with some samples and similarity of drip to juice in composition indicate a generalized failure of drupelet barrier systems (i.e., cell or tonoplast membranes and/or drupelet cuticular membranes) during thawing.

Differences in the extent of drip loss between red and black subsamples sometimes were observed. In such cases, the black-colored fruit, which were riper than the red based on their higher values of SS:A (22), tended to have increased drip losses. Fruit size, as measured by the mean fruit weight (sample weight/number of fruits in sample), had no apparent effect on drip

Table 1. Effect of clone and fruit color on the extent of drip loss and drupelet collapse in thawing thornless blackberries.

		1983				1984			
Clone	Subsample color[z]	SS:A[y]	Mean fruit wt (g)	Drip loss (%)[x]	Drupelet collapse	SS:A	Mean fruit wt (g)	Drip loss (%)	Drupelet collapse
Black Satin	Red	4.9	4.5	33.5	Severe	6.8	4.8	9.0	None
	Black	11.1	4.5	30.7	Severe	7.7	4.4	9.2	None
Chester	Red	4.7	6.2	27.7	Slight	5.5	4.3	7.2	None
	Black	17.2	6.2	36.9	Moder-ate	10.5	4.6	15.9	None
Dirksen Thornless	Red	6.6	4.9	33.2	Severe	7.4	3.9	1.7	None
	Black	20.3	4.5	33.1	Severe	11.8	4.1	5.8	None
Hull Thornless	Red	7.3	5.7	24.0	Severe	11.1	5.0	3.1	None
	Black	24.2	5.8	26.9	Severe	14.6	5.0	6.7	None
Smoothstem	Black	21.7	3.6	23.1	Severe	12.4	4.3	20.1	Severe
C-33	Red	6.6	6.0	14.5	None	8.1	4.4	11.6	None
	Black	27.6	6.8	23.9	None	---	---	---	---
C-61	Red	5.7	5.2	18.3	None	---	---	---	---
	Black	41.6	4.1	17.2	None	---	---	---	---
C-67	Red	---	---	---	---	4.9	5.6	8.6	None
	Black	---	---	---	---	8.5	6.0	29.7	Moder-ate
64-9-3	Red	4.1	4.7	14.6	None	---	---	---	---
	Black	22.6	3.5	10.8	None	---	---	---	---
64-21-3	Red	---	---	---	---	7.1	4.4	1.0	None
	Black	15.4	5.2	11.8	Slight	9.3	4.7	3.5	None
LSD[w]		4.0	1.0	5.3	---	2.8	0.8	3.8	---
Means[v]	Red	5.9	5.2	23.3	---	7.3	4.6	6.0	---
	Black	21.3	5.0	23.8	---	10.5	4.8	13.0	---
LSD[u]		0.9	0.3	1.4	---	0.9	0.3	1.4	---

[z]Color of fruit in frozen state.
[y]Soluble solids (SS, at 20°C) ÷ titratable acidity (A, percentage of citric acid).
[x]Weight loss × 100 ÷ weight of frozen sample.
[w]Mean separation within columns for individual clones by Bonferroni LSD method, 5% level.
[v]Means include clones listed on table in addition to 'Thornfree', C-57, and C-62 for 1983 red subsamples; C-57 and C-62 for 1983 black subsamples; and C-58 for 1984 black subsamples.
[u]Mean separation within columns for means of all clones over both years by Bonferroni LSD method, 5% level.

Table 2. Composition of drip and expressed juice from thornless blackberry fruits.

Clone	Subsample color[z]	Total anthocyanin (A/ml) Drip	Juice	Soluble solids (% at 20°C) Drip	Juice
Chester	Red	39.9	36.7	6.9	7.8
	Black	37.7	31.0	8.1	9.0
Dirksen Thornless	Red	28.4	23.7	7.7	8.6
	Black	22.9	20.3	9.2	10.0
Hull Thornless	Red	20.5	21.1	10.2	9.4
	Black	21.1	18.1	11.8	12.8

[z]Color of fruit in frozen state.

losses; correlations between these variables for red and black fruits considered together or separately were not statistically significant in either year (correlation coefficients of 0.08, 0.38, 0.32, and 0.62 for 1983 red, 1983 black, 1984 red, and 1984 black, respectively). Wenzel et al. (23) reported that drip losses of thawing strawberries increased with riper fruits; they observed no correlation between drip loss and berry size. Sistrunk (20) also observed greater drip losses with over-ripe than with ripe strawberries, which he attributed to the release of pectic enzymes in the over-ripe fruit.

Many of the thornless blackberry samples compared in 1983 showed excessive drip losses, accompanied by drupelet collapse, during thawing. However, certain selections including C-33, C-61, 64-9-3, and 64-21-3 had relatively small drip losses and retained their fresh appearance during thawing.

Blackberry samples obtained in 1984 had smaller drip losses and showed less drupelet collapse than did the 1983 blackberries (Table 1). The SS:A data suggest that the black subsamples but not the red subsamples were riper in 1983 than in 1984; however, even the red subsamples were subject to greater drip losses in 1983 than in 1984. We can speculate that the increased tendency of the 1983 samples to collapse and produce drip during thawing may have been a reflection of their poor condition at the time of harvest or as a consequence of deterioration during shipment from Beltsville to Philadelphia. Morris et al. (16) reported rapid deterioration in the color, flavor, and wholeness of 'Cherokee' blackberries during postharvest holding. Sistrunk (20) observed that holding strawberries prior to freezing increased the extent of drip losses; holding prior to freezing also increased the water-soluble pectin content and decreased the alkali-soluble pectin content of the thawed fruit.

Because of these differences in thawing behavior between years, all statistical analyses were performed on the separate data sets for each year. However, the same trends observed in 1983 samples were seen in the 1984 blackberries. Drip losses

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Table 4. Correlations between drip loss of thawing blackberries and content of soluble and insoluble Ca and pectin.

Comparison	Correlation coefficients[z] 1983 Red fruit	1983 Black fruit	1983 All fruit	1984 Red fruit	1984 Black fruit	1984 All fruit
Drip loss vs.						
Soluble Ca	0.19	−0.57	−0.02	0.40	−0.53	−0.49
Insoluble Ca	−0.10	−0.07	−0.05	−0.20	−0.67	−0.39
Total Ca	0.17	−0.24	−0.04	0.15	−0.62	−0.50
Insoluble/total	−0.25	−0.00	−0.06	−0.40	−0.21	−0.10
Drip loss vs.						
Soluble pectin	−0.02	−0.23	−0.11	−0.93*	−0.59*	−0.68*
Insoluble pectin	−0.05	−0.04	−0.05	−0.66	−0.86*	−0.74**
Total pectin	0.04	−0.22	−0.14	−0.99**	−0.71	−0.80**
Insoluble/total	0.10	0.09	0.08	0.30	−0.47	−0.26

[z]Significantly different from 0 at 5% (*) or 1% (**) level.

Table 5. Morphological features of epidermis of fresh and frozen thornless blackberries, 1984.

Clone	State examined	Drip loss (%)	Cells/running millimeter of cuticle[z] Layer 1	Layer 2	Layer 3	Depth of cell layers (mm[z]) Layer 1	Layer 2
64-21-3	Fresh	---	19	16	11	46	91
	frozen, black	3.5	25	22	14	32	68
Hull Thornless	Fresh	---	16	5	D[y]	36	82
	Frozen, red	3.1	22	16	14	46	86
	Frozen, black	6.7	30	19	D[y]	36	59
Black Satin	Fresh	---	19	11	11	36	86
	Frozen, red	9.0	41	11	8	23	50
	Frozen, black	9.2	27	10	8	27	64
C-33	Fresh	---	27	14	8	23	50
	Frozen, red	11.6	16	12	11	23	64
	Frozen, black	---	30	14	D[y]	23	64
C-67	Fresh	---	33	27	25	23	46
	Frozen, red	8.6	33	10	10	36	64
	Frozen, black	29.7	22	14	11	32	64

[z]Determined with eyepiece micrometer and ×20 objective.
[y]Disorganized.

thornless blackberries must await further study of the mechanical and barrier properties of the epidermis in fresh and frozen fruits of different cultivars and degrees of ripeness.

Drip loss and accompanying drupelet collapse in thawing thornless blackberries are more extensive in fully ripe than in slightly under-ripe fruit. Fruit size has no effect on the extent of drip losses. Differences in fruit condition may be more important than cultivar in determining drip losses. However, several selections appear to be superior to the named cultivars in thawing behavior.

The extent of drip losses is negatively correlated with the pectin content of the fruit but shows no relationship to Ca content. The capacity of blackberry fruits to withstand thawing without excessive drip loss may be associated with the thickness of the epidermis.

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paralleled drupelet collapse, except with 'Chester' blackberries. In general, drip losses were greater with black fruits than with red fruits within a particular cultivar and were not influenced by fruit size. Among the clones compared in both years, 'Smoothstem' and C-33 (red) were similar while 'Black Satin', 'Chester', 'Dirksen Thornless', 'Hull Thornless', and 64-21-3 were much improved in appearance and drip loss. The exceptionally small drip loss of 64-21-3 and acceptable appearance of 'Chester' in spite of substantial drip losses in both years are especially noteworthy.

Composition differences affecting drip loss and appearance. The clones compared varied considerably in soluble, insoluble, and total Ca content (Table 3). Differences between red and black fruits and also between 1983 and 1984 samples were small and indicated no trends.

Because of the well-known binding of Ca to pectin in plant cell walls (5), we postulated that the insoluble Ca content might be negatively correlated with the tendency of thawing blackberries to drip or collapse. However, there was no indication of any relationship between endogenous soluble or insoluble Ca and drip loss in this study (Table 4). Brady et al. (2) were unable to show a consistent relationship between total, water-extractable, or saline-extractable endogenous Ca and firmness in tomato fruit. However, the application of preharvest sprays containing Ca salts to blackberries and raspberries has been shown to improve fruit firmness following postharvest holding (7, 14). Morris et al. (15) reported that a preprocessing dip in Ca lactate solution increased the firmness of thawed, frozen strawberries but had no effect on drained weight. Stanley (22) has proposed using foliar application of Ca for strawberries to improve the texture of the frozen product.

Pectic substances within cell walls and the middle lamella also might be expected to influence drip and/or drupelet collapse during thawing. Differences in soluble and insoluble pectin among clones, between seasons, and between red and black fruits of blackberry samples generally were small (Table 3). However, the insoluble pectin fraction was consistently greater in 1984 than in 1983 samples. Furthermore, the smallest insoluble pectin values in 1984, 0.15% for 'Chester', 'Smoothstem', and C-67 (data not shown) correspond to the greatest drip losses (15.9%, 20.1%, and 29.7%, respectively). While this trend did not appear in the data for 1983 samples, most of these samples h less insoluble pectin than the 1984 blackberries, as well as greater drip losses.

Correlations between the soluble and insoluble pectin contents and drip loss are shown in Table 4. It is clear that the 1984 samples show significant negative correlations between drip loss and pectin. The lack of such correlations for 1983 samples may be due to their small insoluble pectin content and pronounced tendency to leak. The extent of drupelet collapse in thawing samples did not appear to be related to the pectin content in either year. Sistrunk (20) reported greater amounts of water-soluble pectin and less alkali-soluble pectin in over-ripe strawberries from a late-season harvest, compared to berries harvested earlier. The former also had increased drip losses when frozen and thawed. Woodward (24) observed that strawberry fruits undergoing senescence lost almost all of their insoluble pectic polysaccharides.

Morphological differences among cultivars. The dimensions of epidermal and subepidermal cells of thornless blackberry fruits with greatly different drip loss values were compared to determine whether the tendency to leak was associated with any morphological feature (Table 5). The cell density (cells per running millimeter of cuticle), measured in both fresh and frozen fruits, was highly variable and showed no consistent relationship with fruit ripeness (black vs. red) or with drip loss. With strawberries, cultivars having small cells close to each other were reported to be more resistant to the effects of freezing than those with large cells (6). In fresh but not in frozen blackberry fruit the depth of the epidermal and subepidermal cell layers appeared to be reduced with those cultivars that tended to leak more. These data also suggest some shrinkage of epidermal tissues during freezing and/or thawing (in fixative solution). One would expect fruit with a thick epidermis to be more resistant to collapse during thawing than fruit with a thin epidermis. However, a definitive explanation of variation in the leakage behavior of

Table 3. Effects of clone and fruit color on soluble and insoluble Ca and pectin contents of thornless blackberries.

		1983						1984					
		Calcium ($\mu g.g^{-1}$)			Pectin (%)[x]			Calcium ($\mu g \cdot g^{-1}$)			Pectin (%)[x]		
Clone	Subsample color	Soluble	Insoluble	Total	Soluble	Insoluble	Total	Soluble	Insoluble	Total	Soluble	Insoluble	Total
Black Satin	Red	176	98	274	0.55	0.14	0.69	205	29	234	0.38	0.25	0.63
	Black	173	204	377	0.50	0.17	0.68	167	53	220	0.47	0.23	0.70
Chester	Red	---	---	---	---	---	---	---	---	---	0.40	0.28	0.68
	Black	122	55	177	0.37	0.10	0.47	180	55	235	0.37	0.15	0.52
Dirksen Thornless	Red	122	17	139	0.22	0.16	0.38	163	23	186	0.39	0.22	0.61
	Black	126	26	152	0.31	0.14	0.45	155	47	202	0.34	0.19	0.53
Smoothstem	Black	147	84	231	0.44	0.12	0.56	104	36	140	0.30	0.15	0.45
LSD[z]		56	76	114	0.07	0.04	0.09	69	32	84	0.09	0.07	0.12
Total no. clones	Red	6	6	6	7	7	7	5	5	5	7	7	7
	Black	11	11	11	11	11	11	8	8	8	8	8	8
Mean, all clones	Red	203	52	255	0.37	0.17	0.54	205	51	256	0.41	0.25	0.66
	Black	161	92	253	0.39	0.13	0.52	167	44	211	0.40	0.19	0.58
LSD[y]		21	20	34	0.03	0.02	0.04	21	20	34	0.03	0.02	0.04

[z]Mean separation within columns for individual clones by Bonferroni LSD method, 5% level.

[y]Mean separation within columns for means of all clones over both years by Bonferroni LSD method, 5% level.

[x]Expressed as percentage of galacturonic acid fresh weight.